Evaluation of whole genome sequencing for routine subtyping of *Salmonella* Typhimurium for surveillance and outbreak investigation

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CONTEXT

Subtyping = characterisation of bacterial isolates beyond the species and subspecies level

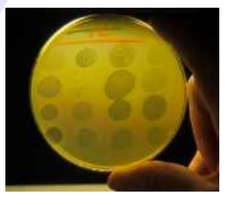
Why?
- Up-to-date diagnosis and surveillance of infectious diseases
- Outbreak: identify a link between the origin of the infection and the human isolate

Case study: *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S.* Typhimurium) and serovar 1,4,[5],12:i:- (*S.* 1,4,[5],12:i:-)

➡ important foodborne pathogen

1) CURRENT SITUATION @WIV-ISP FOR SUBTYPING

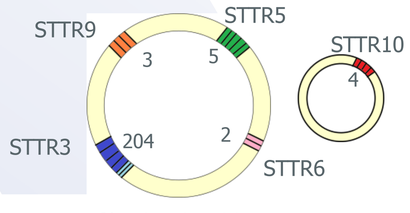
Phage typing



⊕ Very discriminative
Cheap

⊖ High level of expertise
NT and RDNC isolates

MLVA

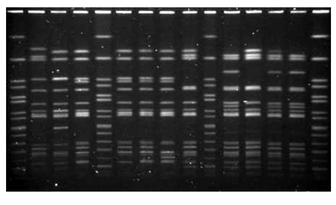


Profile: 3-5-2-4-204

⊕ Profiles easily compared between labs
Rapid

⊖ Too many profiles for surveillance
STTR5, STTR6, STTR10 not stable → outbreak?
(Wuyts *et al.*, 2013; Dimovski *et al.*, 2014)

PFGE



⊕ Medium discriminative

⊖ Slow
Limited reproducibility

➡ None is ideal
Alternative needed

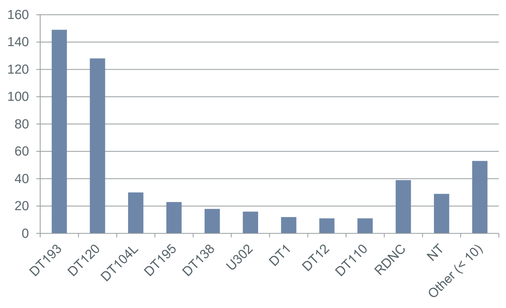
New multiplex method developed with 50 molecular markers in 3 MOL-PCR assays and analysis on Luminex® device
(Wuyts *et al.*, submitted; Wuyts *et al.*, in preparation)

↳ MOL-PCR profile

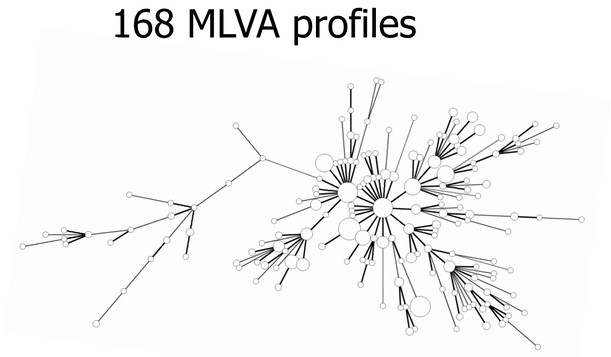
2) VALIDATION OF NEW SUBTYPING METHOD FOR S. TYPHIMURIUM AND S. 1,4,[5],12:i:-

Screening of 519 human *S.* Typhimurium and *S.* 1,4,[5],12:i:- strains isolated in Belgium (2010-2013)

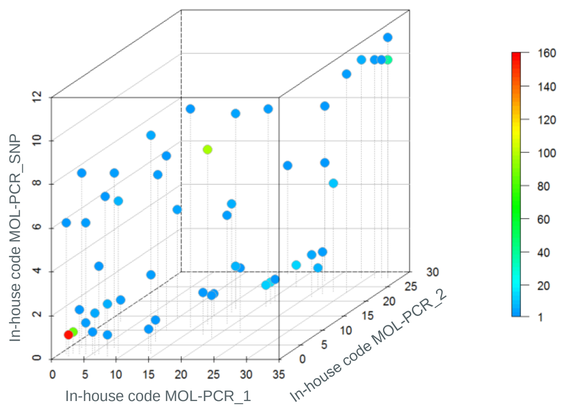
33 phage types



168 MLVA profiles



51 MOL-PCR profiles



50 markers → 51 profiles
more markers ← more profiles

Cost and effort of assay

How many markers?

Other pathogens?

Universally applicable subtyping method

Whole genome sequencing

Human *S.* Typhimurium and *S.* 1,4,[5],12:i:-

		Phage type				
MOL-PCR	MLVA	DT104L	DT120	DT138	DT193	RDNC
2-1-1	3-12-10-NA-211	-	4	-	8	-
	3-12-11-NA-211	-	1	10	-	5
	3-13-11-NA-211	-	-	5	3	2
	3-14-11-NA-211	-	-	-	2	-
2-2-1	3-12-10-NA-211	-	7	1	3	-
	3-12-11-NA-211	-	11	-	3	1
	3-13-11-NA-211	-	1	-	-	-
	3-14-11-NA-211	-	-	-	-	1
30-23-10	3-14-18-14-311	1	-	-	-	-
	3-16-16-13-311	2	-	-	-	-

Outbreak-related → 7 isolates
3 (or 2) isolates → 7×3+2 = 23 isolates
} 30 isolates

3) ONGOING: WHOLE GENOME SEQUENCING FOR ROUTINE SUBTYPING

Data generation: next generation sequencing
- ➡ Illumina in outsourcing
  - ↳ Library type?
  - Coverage?

Data analysis and interpretation
- ➡ Workflow: SNP- or allele-based?
- Definition of distinct subtype?
- Genotype – phenotype?
  - ↳ Antibiotic resistance
- Link with historical data?
  - ↳ Phage type – MLVA

Universal subtyping method
- ➡ Other pathogens
  - ↳ *e.g. S.* Enteritidis

→ Applicable in a routine setting?

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